NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF ISOPRENE

(CAS NO. 78-79-5)

IN F344/N RATS

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

July 1999

NTP TR 486

NIH Publication No. 99-3976

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

R.L. Melnick, Ph.D., Study Scientist
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
R.E. Chapin, Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
R.R. Maronpot, D.V.M.
G.N. Rao, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
R.C. Sills, D.V.M., Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Integrated Laboratory Systems

Battelle Pacific Northwest Laboratories
Conducted studies, evaluated pathology findings

B.J. Chou, D.V.M., Ph.D., Principal Investigator
S.L. Grumbein, D.V.M., Ph.D.
S.E. Rowe, D.V.M., M.S.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
S. Botts, M.S., D.V.M., Ph.D.

Dynamac Corporation
Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

Biotechnical Services, Inc.
Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
J.R. Carlton, B.A.
L.M. Harper, B.S.
A.M. Macri-Hanson, M.A., M.F.A.
W.D. Sharp, B.A., B.S.
S.M. Swift, B.S.

NTP Pathology Working Group
Evaluated slides, prepared pathology report on rats (11 July 1997)

L.L. Lanning, D.V.M, Chairperson
Pathology Associates International
S. Botts, M.S., D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
R. Cattley, V.M.D., Ph.D.
Chemical Industry Institute of Toxicology
V. Geiss, D.V.M., Ph.D.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J.R. Leininger, D.V.M., Ph.D.
National Toxicology Program
S. Platz, D.V.M., Ph.D., Observer
Boehringer Ingelheim
A. Radovsky, D.V.M., Ph.D.
National Toxicology Program
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program
D. Wolfe, D.V.M., Ph.D.
Wolfe Consulting

NTP Pathology Working Group
Evaluated slides, prepared pathology report on kidney step sections in male rats (19 September 1997)

J.B. Nold, D.V.M, Ph.D., Chairperson
Pathology Associates International
J.R. Hailey, D.V.M.,
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J.R. Leininger, D.V.M., Ph.D.
National Toxicology Program
A. Radovsky, D.V.M., Ph.D.
National Toxicology Program
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program

Analytical Sciences, Inc.
Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
S.R. Lloyd, M.S.
N.G. Mintz, B.S.

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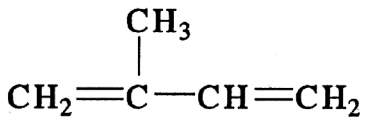
ABSTRACT



ISOPRENE

CAS No. 78-79-5

Chemical Formula: C_5H_8 Molecular Weight: 68.1

Synonyms: Isopentadiene; β-methylbivinyl; 2-methyl-1,3-butadiene

Isoprene, the monomeric unit of natural rubber and naturally occurring terpenes and steroids, is primarily obtained as a by-product of naphtha cracking for ethylene production. It is emitted from plants and trees, has been detected in tobacco smoke and automobile exhaust, and was identified as a major endogenous hydrocarbon in human breath. Isoprene was selected for toxicologic evaluation because of its structural similarity to 1,3-butadiene, a potent, multi-organ, rodent carcinogen, and the potential for human exposure due to its large annual production volume. A previous 26-week inhalation study followed by a 26-week recovery period provided clear evidence of carcinogenic activity of isoprene in male B6C3F$_1$ mice. A similar study in male F344/N rats was inconclusive. Male and female F344/N rats were exposed to isoprene (99% pure) by whole body inhalation for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, mouse bone marrow and peripheral blood cells, and rat lung fibroblasts.

2-Year Study in Rats

Groups of 50 male and 50 female F344/N rats were exposed to 220, 700, or 7,000 ppm isoprene by inhalation, 6 hours per day, 5 days per week, for 105 weeks.

Survival and Body Weights

Survival rates and mean body weights of exposed male and female rats were similar to those of the chamber controls throughout the study.

Urinary Vinyl Lactic Acid Biomarker of Exposure

At 3, 6, 12, and 18 months, the concentrations of vinyl lactic acid normalized to creatinine in the urineincreased with increasing exposure concentration in all exposed groups of male and female rats; however, these increases were not proportional to isoprene exposure concentrations, indicating nonlinear metabolism over this range of exposure concentrations.

Pathology Findings

Exposure-related increases in the incidences of mammary gland fibroadenoma were observed in male rats in all groups. The incidences of fibroadenoma in 7,000 ppm males and in all groups of exposed females were significantly greater than those in the chamber control groups. The incidences of fibroadenoma in all exposed groups of males and females and of multiple fibroadenoma in 7,000 ppm males and in all groups of exposed females exceeded the historical control ranges. In addition, the finding of mammary gland carcinoma in exposed male rats was noteworthy

because this neoplasm rarely occurs in control male rats.

The incidences of renal tubule adenoma in 700 and 7,000 ppm males and of renal tubule hyperplasia in 7,000 ppm males were significantly greater than those in the chamber controls. The severity of kidney nephropathy was slightly increased in 7,000 ppm males when compared to chamber controls.

An exposure-related increase in the incidences of interstitial cell adenoma of the testis was observed in male rats. The incidences of bilateral interstitial cell adenoma and of unilateral and bilateral interstitial cell adenoma (combined) of the testis in 700 and 7,000 ppm males were significantly greater than those in the chamber controls. The incidences of interstitial cell adenoma in 700 and 7,000 ppm males exceeded the historical control range.

Several rare neoplasms including benign astrocytoma, malignant glioma, malignant medulloblastoma, benign meningeal granular cell tumor, and meningeal sarcoma were observed in the brain of exposed female rats. These neoplasms have seldom or never occurred in historical chamber controls.

The incidences of splenic fibrosis in 700 and 7,000 ppm males were significantly greater than that in the chamber control group.

GENETIC TOXICOLOGY

Isoprene was not mutagenic in *S. typhimurium* and did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells with or without exogenous metabolic activation; however, in mice, isoprene induced increases in the frequency of sister chromatid exchanges in bone marrow cells and in the frequency of micronucleated erythrocytes in peripheral blood. The cell cycle duration of proliferating bone marrow cells of mice exposed to 7,000 ppm isoprene was significantly lengthened. No increases in the frequency of chromosomal aberrations were observed in bone marrow cells of male mice after 12 days of exposure to isoprene, and lung fibroblasts of male and female rats exposed to isoprene for 4 weeks showed no increase in the frequency of micronuclei.

CONCLUSIONS

Under the conditions of this 2-year inhalation study, there was *clear evidence of carcinogenic activity** of isoprene in male F344/N rats based on increased incidences of mammary gland fibroadenoma and carcinoma, renal tubule adenoma, and testicular interstitial cell adenoma. There was *some evidence of carcinogenic activity* of isoprene in female F344/N rats based on increased incidences and multiplicity of mammary gland fibroadenoma. A low incidence of rare brain neoplasms in exposed female rats may have been due to exposure to isoprene.

Exposure to isoprene by inhalation for 2 years resulted in increased incidences of renal tubule hyperplasia and splenic fibrosis in male rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Isoprene

	Male F344/N Rats	Female F344/N Rats
Concentrations in air	0, 220, 700, or 7,000 ppm	0, 220, 700, or 7,000 ppm
Body weights	Exposed groups similar to chamber control group	Exposed groups similar to chamber control group
Survival rates	18/50, 16/50, 15/50, 15/50	29/50, 30/50, 28/50, 27/50
Nonneoplastic effects	Kidney: renal tubule hyperplasia (standard evaluation - 0/50, 2/50, 6/50, 8/50; standard and extended evaluations combined - 7/50, 6/50, 13/50, 18/50) Spleen: fibrosis (11/50, 14/50, 24/50, 22/50)	None
Neoplastic effects	Mammary gland: fibroadenoma, multiple (1/50, 1/50, 0/50, 7/50); fibroadenoma, including multiple (2/50, 4/50, 6/50, 21/50); carcinoma (0/50, 1/50, 1/50, 2/50) Kidney: renal tubule adenoma (standard evaluation - 0/50, 2/50, 2/50, 6/50; standard and extended evaluations combined - 2/50, 4/50, 8/50, 15/50) Testis: interstitial cell adenoma, bilateral (20/50, 29/50, 37/50, 48/50); interstitial cell adenoma, including bilateral (33/50, 37/50, 44/50, 48/50)	Mammary gland: fibroadenoma, multiple (7/50, 12/50, 19/50, 17/50); fibroadenoma, including multiple (19/50, 35/50, 32/50, 32/50)
Uncertain findings	None	Brain: benign astrocytoma (0/50, 0/50, 1/50, 0/50); malignant glioma (0/50, 0/50, 0/50, 1/50); malignant medulloblastoma (0/50, 0/50, 0/50, 1/50); meninges, benign granular cell tumor (0/50, 1/50, 0/50, 1/50); meninges, sarcoma (0/50, 1/50, 0/50, 1/50)
Level of evidence of carcinogenic activity	Clear evidence	Some evidence

Genetic toxicology

Salmonella typhimurium gene mutations:	Negative in strains TA98, TA100, TA1535, and TA1537 with and without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Mouse bone marrow *in vivo*:	Positive
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Mouse bone marrow *in vivo*:	Negative
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo*:	Positive
Lung fibroblasts:	Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on isoprene on 10 December 1997 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Gary P. Carlson, Ph.D., Chairperson
School of Health Sciences
Purdue University
West Lafayette, IN

A. John Bailer, Ph.D.
Department of Mathematics and Statistics
Miami University
Oxford, OH

Steven A. Belinsky, Ph.D., Principal Reviewer
Inhalation Toxicology Research Institute
Kirkland Air Force Base
Albuquerque, NM

James S. Bus, Ph.D.
Health and Environmental Sciences
Dow Chemical Company
Midland, MI

Linda A. Chatman, D.V.M.
Pfizer, Inc.
Groton, CT

John M. Cullen, Ph.D., V.M.D., Principal Reviewer
Department of Microbiology, Parasitology, and Pathology
College of Veterinary Medicine
North Carolina State University
Raleigh, NC

Susan M. Fischer, Ph.D.
M.D. Anderson Cancer Center
University of Texas
Smithville, TX

Thomas L. Goldsworthy, Ph.D.
Integrated Laboratory Systems
Research Triangle Park, NC

Irma Russo, M.D.
Fox Chase Cancer Center
Philadelphia, PA

Special Reviewers

Stephen S. Hecht, Ph.D.
University of Minnesota Cancer Centers
Minneapolis, MN

Michele Medinsky, Ph.D., Principal Reviewer
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Jose Russo, M.D.
Fox Chase Cancer Center
Philadelphia, PA

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 10 December 1997, the draft Technical Report on the toxicology and carcinogenesis studies of isoprene received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.L. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of isoprene by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats. A previous NTP 26-week inhalation study followed by a 26-week recovery period had provided clear evidence of the multiple-site carcinogenicity of isoprene in male B6C3F$_1$ mice. The proposed conclusions for the 2-year study were *clear evidence of carcinogenic activity* in male F344/N rats and *some evidence of carcinogenic activity* in female F344/N rats.

Dr. Melnick discussed the metabolism of isoprene and compared the neoplasm responses and metabolism of isoprene with two close structural analogues, 1,3-butadiene and chloroprene, from previous 2-year NTP studies. Dr. Melnick then described a physiologically based pharmacokinetic model that was developed for isoprene and how it was used to evaluate dose-response relationships for neoplasm formation at the different sites.

Dr. Belinsky, a principal reviewer, agreed with the proposed conclusions.

Dr. Medinsky, the second principal reviewer, agreed with the proposed conclusions. She said the structure-activity comparisons made with the analogues maximized the usefulness of the data collected on the three chemicals. Dr. Medinsky noted the use of pharmacokinetic data to obtain a more refined dose metric and a more refined demonstration of the changes in neoplasm response with respect to dose. She asked why the authors believed the parent substance, isoprene, might be involved directly in neoplasm formation. Dr. Melnick said the relationship in the kidney seems to be driven by the mono- or diepoxide intermediate, but in the mammary gland of male rats, there may be some contribution from the parent because of the greater response at the highest exposure concentration.

Dr. Cullen, the third principal reviewer, agreed with the proposed conclusions.

Dr. A.P. Leber, Goodyear Tire and Rubber Company, representing the International Institute for Synthetic Rubber Producers, said that he had specific comments on the neoplasms reported. With regard to rat renal tubule neoplasms, he said that the dimer of isoprene is limonene, which is known to be associated with α2u-microglobulin accumulation and renal neoplasms in rats. [Ed. Note: protein droplet accumulation was not observed in the male rat kidney in the current study]. In his view, the low incidence of carcinoma and lack of indication of a progression of male mammary gland neoplasms to malignancy supported only *some evidence*, as would also be the case with the testicular neoplasms. For the mammary gland neoplasms in female rats, he said that *equivocal evidence* would be appropriate.

Dr. Belinsky moved that the Technical Report on isoprene be accepted with revisions discussed and the conclusions as written for male rats, *clear evidence of carcinogenic activity*, and for female rats, *some evidence of carcinogenic activity*. Dr. Medinsky seconded the motion, which was accepted by six yes votes to one no vote (Dr. Goldsworthy) with one abstention because of company affiliation (Dr. Bus).